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THE EXPRESSION AND TRAFFICKING OF AQUAPORIN-2 IN TWO DIFFERENT KIDNEY EPITHELIAL CELL MODELS IS OSMOLALITY DEPENDENT

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The water permeability of the rat kidney inner medullary collecting duct (IMCD) is regulated by the antidiuretic hormone vasopressin (AVP) *via* the insertion of the water channel aquaporin-2 (AQP2) into the apical membrane of IMCD principal cells. Hormone binding induces a rise in intracellular cAMP, which regulates both AQP2 biosynthesis (long-term regulation) and the trafficking of AQP2 bearing vesicles to the cell membrane (short-term regulation). The aim of the presented study was to investigate the influence of extracellular osmolality on AQP2 expression and trafficking under *in vitro* conditions using MDCK1 cells stably transfected with AQP2 (WT-10 cells; 1) and primary cultured IMCD cells (2). In WT-10 cells AQP2 the expression is governed by a strong viral promoter, while in primary cultured IMCD cells high AQP2 expression levels were achieved by dbcAMP (500 µM) supplementation of the culture media.
Western blot analyses of cell membrane preparations revealed an increased AQP2 protein expression for cells cultivated in media with elevated osmolality (600 mOsm/kg). In addition AQP2 expression was further increased by acidification of the culture media (pH = 6.4). Immunofluorescence studies demonstrated the osmolality dependence of the redistribution of AQP2 to the basolateral or the apical membrane compartments in WT-10 cells.
Taken together the results indicate an osmolality- and pH-sensitive regulation of AQP2 expression superimposed on the known vasopressin-dependent regulation. These findings may prove useful to develop concepts for the treatment of diuretic states.

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SIZE DISTRIBUTION AND KINETICS OF EXOCYTOTIC INSERTION OF AQUAPORIN-2 BEARING VESICLES INTO THE CELL MEMBRANE OF PRIMARY CULTURED RENAL PRINCIPAL CELLS

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The water channel aquaporin-2 (AQP2) is redistributed to the plasma membrane of inner medullary collecting duct (IMCD) cells when the cells are stimulated with arginine vasopressin (AVP). Applying the patch clamp technique to primary cultured IMCD cells we recently demonstrated a specific AVP-induced cAMP dependent increase of cell membrane capacitance, suggesting insertion of vesicles into the cell membrane. The aims of the present investigation were to study (I) in detail the size of vesicles by capacitance measurements and electron microscopy, and (ii) the kinetics of the exocytotic processes by flash photolysis of a newly synthesized caged cAMP analogue (BCMCM-8Br-cAMP).
The analysis of capacitance recordings obtained from IMCD cells stimulated intracellularly with 300 µM cAMP through the patch pipette revealed a multimodal frequency distribution of resolved capacitance step sizes with peaks of about 7 fF and multiples of that value. The occurrence of multiple peaks might be explained by the preformation of large vesicles from smaller once. In the case of AVP stimulation only one peak around 6 fF occurred in the frequency distribution. Electron microscopy supported the results from electrophysiological measurements.
Irradiation of BCMCM-8Br-cAMP with UV light from a flash lamp rapidly liberates 8Br-cAMP and the photoproduct BCMCM-OH. The strong fluorescence of BCMCM-OH allowed the monitoring of the liberation process in living IMCD cells. The rapid enhancement of 8Br-cAMP by flash photolysis increased the capacitance of IMCD cells after a delay of a few seconds. In contrast to neuronal cells, fast capacitance increases within milliseconds were only rarely observed. From these data we conclude that only a very small pool of readily releasable vesicles are present in IMCD cells.

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LIDOCAINE BLOCK IS LINKED TO MOLECULAR MOTIONS OF THE Na^+ CHANNEL OUTER VESTIBULE

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The local anesthetic lidocaine is considered to bind with high affinity to the inactivated state of Na^+ channels. Adult rat skeletal muscle Na^+ channels (µ1), when expressed in *Xenopus* oocytes, recover from inactivation with four time constants (τ), suggesting that channels assume at least four distinct inactivated conformations, namely fast inactivation (I-F; $\tau \sim 2$ ms), intermediate inactivation (I-M; $\tau \sim 700$ ms), slow inactivation (I-S; $\tau \sim 3$ s), and ultra-slow inactivation (I-US; $\tau \sim 100$ s). The differential role of these inactivated conformations with regard to lidocaine action is poorly understood.
In the present study we examined a possible interaction of lidocaine with I-US. Entry into I-US was promoted by the outer pore mutation µ1-K1237E. Binding to the outer pore of a partially blocking peptide (µ-conotoxin GIIIA-R13Q) substantially reduced the number of channels recovering from the I-US state, indicating that I-US occurred via a structural rearrangement of the outer vestibule. In order to assess the interaction of lidocaine with I-US, µ1-K1237E channels, heterologously expressed in *Xenopus* oocytes, were depolarized by a 300 s conditioning pulse to -20 mV. Thereafter, recovery from inactivation was monitored by 10 ms test pulses to -10 mV, applied at 20 s intervals from a holding potential of -120 mV. During a drug-free control 87 ± 5.2 % of channels recovered from I-US, i.e. with a time constant of ~100 s. Superfusion with 1 mM lidocaine significantly reduced recovery from I-US to 31.7 ± 4 % (n= 5, P<0.01).
Coexpression of the Na^+ channel ß1-subunit with the α-subunit has been shown to decrease I-F, I-M, and I-S, but not I-US (Todt et al., *Biophys. J.* 76, 1335; Nuss et. al., *J.Gen.Physiol.* 106, 1171). Thus, if the lidocaine induced reduction of the number of channels recovering from I-US had resulted from an interaction with I_F, I_M, or I_S, coexpression with the ß1 subunit would have altered the lidocaine mediated decrease of I-US. However, coexpression of µ1-K1237E α-subunits with ß1 did not change the lidocaine induced decrease in I-US, suggesting a selective interaction of lidocaine with this kinetic state. In molecular terms we interpret the results to indicate that the lidocaine binding site may be functionally coupled to the outer vestibule of the Na^+ channel.

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LONG-TERM TREATMENT WITH THE NHE-1 INHIBITOR CARIPORIDE EXTENDS THE NORMAL LIFESPAN OF WISTAR KYOTO RATS

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We investigated the outcome of lifelong treatment with the sodium proton exchange-1 (NHE-1) inhibitor, cariporide in young normotensive Wistar-Kyoto rats (WKY rats). In addition to the primary end point lifespan, surrogate parameters such as cardiac left ventricular hypertrophy, cardiac function and metabolism, and endothelial function were studied. Furthermore, heart, kidney, skeletal muscle and eyes were histomorphologically investigated.
Ninety, one-month-old, WKY rats were randomized into two groups: placebo treated (n=45) and cariporide treated (n=45) via the food (0.3% in the chow). Surrogate parameters were conducted after 30 months, when about 80% of the placebo group had died.
Lifelong treatment with cariporide highly significantly extented lifespan of the animals from 30 to 39 months which was correlated with a delayed occurrence of cancer. Age-related cardiac hypertrophy observed in placebo treated animals was fully prevented by cariporide treatment. This was reflected by improved function and metabolism when the hearts were investigated in the isolated working heart mode. Age-related endothelial dysfunction measured in isolated aortic rings from the animals was significantly improved by NHE-1 inhibition.
Age-related changes such as heart muscle fibrosis, muscular dystrophy, tubulo-interstitial lesions in the kidney and retina atrophy were drastically reduced by cariporide treatment.
Lifelong treatment of normotensive WKY rats with the NHE-1 inhibitor cariporide extended lifespan from 30 to 39 months. This lifespan extension correlated with a prevention of left ventricular hypertrophy, cardiac and vascular dysfunction and age-related changes in investigated organs. Furthermore, the occurrence of cancer was significantly delayed, which corresponded to the life-span extension.

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